

Respectfully submitted,

MERCHANT & GOULD P.C.
P.O. Box 2903
Minneapolis, Minnesota 55402-0903
(612) 332-5300

Date: Sept. 20, 2001

Katherine M. Kowalchuk
Katherine M. Kowalchuk
Reg. No. 36,848
KMK:sab



VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification

Paragraph beginning at page 1 after the title has been amended as follows:

Cross Reference to Related Application

This application claims benefit of U.S. Provisional Application Serial No. 60/243,913, filed October 27, 2000, which application is incorporated herein by reference.

Paragraph beginning at line 21, page 6, has been amended as follows:

Figure 7 shows an amino acid sequence of recombinant human TPO (SEQ ID NO:13).

Paragraph beginning at line 17, page 19, has been amended as follows:

An immunodominant epitope of human thrombopoietin includes amino acids 318 to 332 and has the following sequence (represented in single letter code):

LNTSYTHSQNLSEQ (SEQ ID NO:1)

Paragraph beginning at line 7, page 22, has been amended as follows:

Analysis of the amino acid sequence of human thrombopoietin resulted in the identification of a predicted immunodominant epitope using the service provided by Epivax, Inc. Fourteen out of 15 amino acid residues of the immunodominant peptide identified using antibodies from naïve patients showed 100% homology with the 14 residues at the C-terminal of the 20 amino acid region predicted by EpiVax. The sequence of the predicted immunodominant epitope has an amino acid sequence identical to amino acid residues 312 to 331:

TPTSPLLNTSYTHSQNLSQE (SEQ ID NO: 2)

Paragraph beginning at line 8, page 40, has been amended as follows:

A specific example of a modified polypeptide is a modified thrombopoietin having reduced immunogenicity while retaining substantial therapeutic activity. An

immunodominant epitope in native sequence human thrombopoietin is a c-terminal peptide including amino acids 318 to 332 :

LNTSYTHSQNLSQEG (SEQ ID NO.: 1)

The table beginning at line 14, page 50, with the following rewritten paragraph:

Table 2

Synthetic TPO c-terminal epitope peptides
and corresponding rabbit anti-peptide antibodies

peptide sequence (length)	antibody ID #
154-170(17)	24
175-190(16)	48
195-211(17)	28
18-234(17)	19
244-259(16)	17
258-268(11)	49
268-283(16)	16
296-311(16)	51
318-332(15)	15

The peptides have the following sequences:

154- 170	RAPPTTAVPSRTSLVLT (<u>SEQ ID NO: 3</u>)
175-190	PNRTSGLLETNFTASA (<u>SEQ ID NO: 4</u>)
195-211	SGLLKWQQGFRAKIPGL (<u>SEQ ID NO: 5</u>)
218-234	SLDQIPGYLNRIHELLN (<u>SEQ ID NO: 6</u>)
244-259	SRRTLGA PDISSGTSD (<u>SEQ ID NO: 7</u>)
258-268	SDTGSLPPNLQ (<u>SEQ ID NO: 8</u>)

268-283 QPGYSPSPTHPPTGQY (SEQ ID NO: 9)
 296-311 VVQLHPLLDPDSAPTP (SEQ ID NO: 10)
 318-332 LNTSYTHSQNLSQEG (SEQ ID NO: 1)

Paragraph beginning at line 12, page 52, has been amended as follows:

The entire linear amino acid sequence of human recombinant TPO was provided to EpiVax, Inc. for analysis and prediction of immunodominant epitopes. The results from EpiVax, Inc. identified 3 regions within the c-terminal epitope that have class II MHC binding motifs. One of these predicted epitope regions (identical to amino acids 312-331) is shown:

TPTSPLLNTSYTHSQNLSQE (SEQ ID NO: 2)

Paragraph beginning at line 17, page 53, has been amended as follows:

A cDNA corresponding to the hTPO entire open reading frame was obtained by PCR using the oligonucleotide primes of the following Table.

CHO Expression Vector PCR Primers

<p>Cla.FL.F2 5' ATC GAT ATC GAT AGC CAG ACA CCC CGG CCA G 3'</p> <p>(<u>SEQ ID NO:11</u>)</p>
<p>ORF.Sal 5' AGT CGA CGT CGA CGT CGG CAG TGT CTG AGA ACC 3'</p> <p>(<u>SEQ ID NO:12</u>)</p>

PRK5-*hmpl* I was used as template for the reaction in the presence of pfu DNA polymerase (Stratagene). Initial denaturation was for 7 min. at 94°C followed by 25 cycles of amplification (1 min. at 94°C, 1 min. at 55°C and 1 min. at 72°C). Final extension was for 15 min. at 72°C. The PCR product was purified and cloned between the restriction sites ClaI and Sall of the plasmid pSV15.ID.LL to obtain the vector pSV15.ID.LL.MLORF. The sequence of the construct was verified.